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Impact of physical and biological factors on susceptibility of *Tribolium castaneum* and *Tribolium confusum* (Coleoptera: Tenebrionidae) to new formulations of hydroprene[☆]

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Abstract

Three separate experiments were conducted to: (1) evaluate two new commercial formulations (202-080 and 202-084) of the insect growth regulator hydroprene, (2) determine residual efficacy of hydroprene-treated concrete stored at different environmental conditions, and (3) assess the impact of accumulated flour on residual efficacy. In the first test, late instars of *Tribolium castaneum* (Herbst), the red flour beetle, and *Tribolium confusum* (du Val), the confused flour beetle, were exposed on concrete treated with hydroprene. At 40% relative humidity (r.h.), there was no difference between species regarding the percentage of individuals that stopped development in the larval stage (arrested larvae), but at 75% r.h. there were more arrested *T. castaneum* than *T. confusum* in all treatments except the low rate of formulation 202-084. No adult *T. castaneum* lived after emergence (live adults) at either relative humidity, but the percentage of live adult *T. confusum* ranged from 1.0% to 41.0%, depending on treatment. In the second test, late instars of *T. confusum* were exposed at 6 and 12 weeks post-treatment on concrete treated with the two experimental formulations and stored under different environmental conditions. At 6 weeks there was no difference between formulations. At 12 weeks, fewer live adults and more dead emerged adults with gross morphological deformities were found on concrete treated with formulation 202-084 and stored at 32°C, 75% r.h. compared to other treatment combinations. In the final experiment, wheat flour was added to treated concrete for 5 weeks before the bioassays were conducted with late-instar *T. confusum*. There were few live adults produced in the initial bioassays, and dead adults with gross morphological deformities ranged from 83.1% to 97.6%. However, in bioassays conducted with late-instar larvae at 6 weeks, most adults eventually emerged with few deformities. The presence of the flour apparently compromised residual control and the hydroprene was no longer effective. In summary, the new hydroprene formulations were

[☆] This paper reports the results of research only. Mention of a proprietary product or chemical does not constitute a recommendation or endorsement by the US Department of Agriculture.

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equivalent to the registered product Gentrol[®]. *Tribolium confusum* was less susceptible than *T. castaneum*, and residual control of *T. confusum* on a clean surface without flour lasted about 6–12 weeks.

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Keywords: Hydroprene; *Tribolium castaneum*; *Tribolium confusum*; Treated surfaces; Control

1. Introduction

Insect growth regulators (IGRs) are chemicals which mimic hormones that control molting in insects and thereby disrupt development. When cockroach nymphs are exposed to IGRs, common results include arrest in the nymphal stage, incomplete adult emergence, and adults with various morphological deformities (King and Bennett, 1988, 1991; Atkinson et al., 1992). Studies with cockroaches showed that even if adults successfully emerge after being exposed in the immature stages to IGRs, the exposure often produces sterility and reproductive inhibition (King and Bennet, 1989).

Oberlander et al. (1997) reviewed many studies involving IGRs and stored product-insects, and there are other published studies as well (McGregor and Kramer, 1976; Amos et al., 1977; Gupta and Mkhize, 1983; Mian and Mulla, 1982a, b, 1983; Rup and Chopra, 1984; El-Sayed, 1988). Most of the published research involved exposure of eggs, 1st instars, or adults on treated grain, in diet, or in glass vials, with subsequent measurements of progeny production. Morphological effects and reduced fecundity in adults have been reported from some of these studies with stored-product insects (Amos et al., 1977; Hoppe, 1981).

Many stored-product insects occur not only in raw grains, but also in mills, processing plants, food warehouses, and other indoor areas, and there is comparatively little research with IGRs that is applicable to insect control programs in those areas. Insecticides are used in these areas as either aerosols or residual crack and crevice and surface sprays, and when the insecticides are applied to a flooring surface such as concrete, insects are directly exposed on that treated surface. Many insecticides degrade quickly on concrete because of its high alkalinity (Okwelogu, 1968). In a recent test late-instar larvae of *Tribolium castaneum* (Herbst), the red flour beetle, and *Tribolium confusum* (du Val) were directly exposed on concrete treated with hydroprene (Arthur, 2001). Larvae often failed to molt to the pupal stage, and adults that did emerge were usually morphologically deformed, and quickly died. In addition, *T. castaneum* appeared to be more susceptible to hydroprene than *T. confusum*.

Currently there is renewed interest in developing reduced-risk low toxicity chemicals, including new formulations of IGRs, to replace older conventional products used in many agricultural systems, including post-harvest protection in areas where food is stored. Expanded use of these IGRs could also be facilitated by identifying physical and biological factors that can compromise efficacy when IGRs are used as a surface treatment. Three separate experiments were conducted in this study to: (1) evaluate susceptibility of *T. castaneum* and *T. confusum* to new experimental formulations of hydroprene, at levels of 40% and 75% relative humidity (r.h.), (2) assess the impact of residual degradation on efficacy of hydroprene, and (3) determine effects on efficacy resulting from repeated coverings of flour on a surface treated with hydroprene. The specified relative humidity levels were chosen to represent an upper and lower

end point of the range commonly encountered inside grain storage structures during a typical year.

2. Materials and methods

2.1. Experiment 1. Evaluation of new hydroprene formulations: impact of relative humidity and susceptibility of *T. castaneum* and *T. confusum*

This test consisted of six treatments; untreated concrete, concrete treated with the label rate of the Gentrol[®] formulation of hydroprene EC (1.9×10^{-3} mg [AI]/cm²), and concrete treated with 0.85 and 1.9×10^{-3} mg [AI]/cm² of experimental hydroprene formulations 202-080 and 202-084 (low and high rates). Five replicates of the test were conducted at 27°C, 40% and 75% r.h., using 4-week-old *T. castaneum* larvae and 4-week-old *T. confusum* larvae, and each replicate was done separately. Insects were obtained from pesticide-susceptible cultures maintained at approximate rearing conditions of $27 \pm 2^\circ\text{C}$, $60 \pm 5\%$ r.h., no light:dark cycle. Two separate humidity chambers were created by filling 26 by 36.5 by 15 cm plastic boxes, with waffle-type grids cut to fit the bottom, with about 750 ml of either saturated K₂CO₃ or NaCl to maintain the relative humidity at either 40% or 75%, respectively (Greenspan, 1977).

For the first replicate, 24 (six treatments \times two species \times two relative humidity levels) individual treatment units were constructed by partially filling standard 100 \times 15 mm plastic Petri dishes with ready-mix concrete slurry, then allowing the concrete to dry on a laboratory countertop for several days, as described for previous tests (Arthur, 2001). The field spray rate for Gentrol[®], equivalent to 0.17 ml of formulated material for the area of the Petri dish (62 cm²), was used for the four concrete arenas treated with Gentrol[®] (two species, two relative humidity levels), and for each of the four arenas treated with the low and high rates of the two experimental hydroprene formulations. Untreated controls were treated with the same volume of tap water. The insecticide treatments were applied using a Badger 100 artists' airbrush (Franklin Park, IL, USA) to mist water for the untreated controls or the insecticide solutions directly on the concrete arenas.

After the concrete arenas were treated with the hydroprene concentrations, they were allowed to dry for 1 day inside a laboratory hood. Twenty mixed-sex 4-week-old late-instar larvae of each species were put in each of two separate arenas from each of the insecticide treatments and the untreated controls, along with approximately 500 mg of wheat flour. One set of arenas containing each species and all six treatments was placed on the waffle grid in the 40% r.h. chamber. The other set of arenas was placed in the same manner on the grid in the 75% r.h. chamber, and both boxes were put inside an incubator set at 27°C.

At 27°C, larvae of both beetle species would normally pupate within a week and emerge as adults after another week. The concrete arenas were held inside the incubator for 5 weeks after the larvae were introduced, which was 3 weeks beyond the time they would normally emerge as adults in the absence of insecticide treatment. They were then removed from the incubator, and in each arena the number of larvae and pupae were recorded, along with the number of adults that had died after they emerged (hereafter referred to as dead adults). These dead adults were further classified as having either: (1) wing deformities, which were defined as twisted, incomplete, and unsclerotized wings, or (2) gross morphological deformities, which were defined as wing

deformities plus incomplete emergence, presence of pupal characters, incomplete sclerotization, and missing body parts. The number of adults that had remained alive after emergence (hereafter called live adults) with morphological deformities was also recorded.

Upon conclusion of the first replicate, all treatment and exposure procedures were repeated in succession for each of the four additional replicates. Data for all five replicates were analyzed with insecticide treatment, insect species, and relative humidity as main effects. Response variables were the percentage of each species that remained in either the larval or pupal stage 5 weeks after they were first exposed on the treated concrete, the percentage of live adults, and the percentage of dead adults that had either gross morphological deformities or wing deformities. Statistical analyses were performed using the Means and General Linear Model Procedures of the Statistical Analysis System (SAS Institute, 2001). Unless otherwise noted, significance was determined at the 0.05 level.

2.2. Experiment 2. Impact of environmental conditions on residual efficacy of hydroprene against *T. confusum*

The results of experiment 1 showed there was little or no difference between the efficacy of the Gentrol[®] formulation of hydroprene and equivalent rates of the experimental hydroprene formulations 202-080 and 202-084, so further tests were conducted with the experimental compounds only. Also, late-instar *T. castaneum* were much more susceptible to hydroprene than late-instar larvae of *T. confusum*, therefore these additional tests were conducted using the more tolerant *T. confusum*.

Trials were conducted first with hydroprene formulation 202-080, using the application rate of 1.9×10^{-3} mg [AI]/cm². A total of 120 concrete treatment arenas were constructed as described for experiment 1. For formulation 202-080, the test was designed with the following factors: two storage temperatures, 22°C and 32°C; two relative humidity levels, 40% and 75%; and five residual bioassay intervals, 0, 6, 12, 18, and 24 weeks after treatment. There were five treated replicates and an untreated control replicate for each factor. Insecticide solutions were formulated for each of the five treated replicates, and each replicate was treated separately and untreated controls were sprayed with tap water, as described for experiment 1 (20 treated arenas and four untreated controls per replicate). Four new relative humidity boxes containing salt solutions, two for 40% r.h. and two for 75% r.h. were also created as described for experiment 1, along with two other boxes with saturated NaBr to maintain the relative humidity at approximately 57% (Greenspan, 1977).

One day after treatment, 20 treated and four untreated arenas were put in each of the four humidity boxes, and one 40% r.h. box and one 75% r.h. box was put inside each of two temperature incubators set at 22°C and 32°C. The remaining 20 treated and four untreated arenas were kept aside for the 0-month residual treatment to provide baseline data (two temperatures, two relative humidity levels, five treatments, and one untreated control). The actual residual bioassays were conducted at 27°C and 32°C, 57% r.h., to provide optimal conditions for *T. confusum*. Twenty mixed-sex late-instar *T. confusum* larvae, along with 500 mg of wheat flour, were exposed on each arena, and each arena was covered and placed in the 57% r.h. box, which was in turn set inside the 27°C incubator. After 6 weeks, the arenas were removed from the incubator, and the individual *T. confusum* were classified as described for experiment 1 and the numbers in each category were recorded.

Bioassays for the 6-week residual trial were conducted by removing a set of five treated concrete arenas and one untreated arena from each of the four humidity boxes, two from each temperature incubator, and exposing 20 mixed-sex late-instar *T. confusum* larvae, along with 500 mg of wheat flour, in each arena. These arenas were also placed in the 57% r.h. box, set inside the 27°C incubator, held for 6 weeks, and the assessment procedures were repeated and the insects discarded. Bioassays were repeated again for the 12-week residual trial, following procedures described above. After the assessments were made on these arenas, the test was discontinued because of the large number of live adults found in the treated arenas from the 12-week bioassays.

The trial for hydroprene formulation 202-084 was initiated 3 weeks after the start of the trials for formulation 202-080. A second set of 120 concrete arenas was constructed, following all procedures as described for the test with formulation 202-080 detailed in the preceding paragraphs. Treated and untreated arenas were held aside for the 0-week bioassays, and the remainder stored for 6, 12, 18, and 24 weeks, and bioassayed following the same procedures as described for formulation 202-080. In this trial with formulation 202-084, the residual bioassays for 18 and 24 weeks were also eliminated because of excessive live adults in the treatments after the 12-week bioassays.

Data for the exposures on the treated concrete were analyzed with formulation, temperature and relative humidity at which the concrete was stored (storage environment), and residual bioassay (0, 6, 12, or 18 weeks) as main effects. Response variables and statistical analyses were as described for experiment 1.

2.3. Experiment 3. Residual susceptibility of *T. confusum* to hydroprene on concrete repeatedly covered with flour

In this test all residual bioassays were conducted on the same set of treated concrete arenas rather than using new arenas for each bioassay as was done for experiment 2. In addition, because the same set of arenas was used throughout the test, these arenas were not stored at different temperatures and relative humidity levels as described in experiment 2. Instead, the bioassay was a multi-factorial design in which *T. confusum* was exposed at two temperatures, 27°C and 32°C, 57% and 75% r.h., at four bioassay intervals of 0, 6, 12, and 18 weeks post-treatment, with five treated replicates and one untreated replicate for each factor. Two new humidity boxes were created that contained saturated NaCl to maintain humidity at 75%, as described for experiment 1, and two new humidity boxes were created to maintain 57% r.h. as described in experiment 2.

Tests were initiated first with formulation 202-080 by creating 24 new concrete arenas and treating 20 at the rate of 1.9×10^{-3} mg [AI]/cm² of hydroprene, and treating the remaining 4 with tap water, which were the untreated controls for each factor. Twenty mixed-sex late-instar *T. confusum* were put in each arena, along with 500 mg of flour, and five of the treated arenas and one untreated arena was put in each of the four humidity boxes. One box at 57% r.h. and one box at 75% r.h. was put in each of two temperature incubators set at 27°C and 32°C. After 6 weeks, the concrete arenas were removed from the humidity boxes and the temperature incubators, and insects were classified as described for the previous experiments. The flour was removed from the arenas and discarded.

For the next series of bioassays at 6 weeks, 20 mixed-sex late-instar *T. confusum* larvae were put in each of the same dishes that had been used for the 0-week bioassays, along with 500 mg of

wheat flour. The arenas were put in the respective humidity boxes, and then the boxes were in turn put into the temperature incubators. After 6 weeks, the dishes containing the flour and the concrete arenas were removed from the incubators, the various life stages were sifted from the flour and tabulated as previously described. The arenas were cleaned, and the entire exposure process was repeated again at 12 weeks post-treatment.

As in experiment 2, the trials with hydroprene formulation 202-084 were initiated 3 weeks after the trial with formulation 202-080, following the same procedures as outlined in the previous paragraphs. This experiment was also discontinued after the assessment of the 12-week bioassays because of the large number of live adults in the treatments. Data were analyzed with exposure temperature, relative humidity, and residual bioassay as main effects. Response variables and statistical analysis was as described for experiment 1.

In all three experiments, virtually all of the larvae exposed on untreated concrete emerged as adults well before the conclusion of the test and all were still alive, and none of these adults were deformed in any manner. Untreated controls were therefore eliminated from further statistical analysis, and each of the response variables were analyzed in succession. Also, at the conclusion of the test there were few beetles of either species that were in the pupal stage, regardless of treatment, therefore this variable was eliminated from the analysis of the treatments.

3. Results

3.1. Experiment 1. Evaluation of new hydroprene formulations: impact of relative humidity and susceptibility of *T. castaneum* and *T. confusum*

All main effects and most interactions were significant with respect to the percentage of exposed beetles that stopped development in the larval stage (arrested larvae) and for live adults emerging from these larval exposures (Table 1). Main effects were significant for the percentage of dead adults with gross morphological deformities; however, the percentage of adults with wing deformities only was significant for just the main effects treatment and species (Table 1).

Although significance varied among the treatments regarding arrested larvae, there were no differences among the Gentrol[®] formulation and the two experimental formulations of hydroprene applied at the same rate as Gentrol[®] ($P \geq 0.05$). At 40% r.h., there was no statistical difference between species even though the means for *T. castaneum* were nearly double those of *T. confusum* in treatments 1, 3, and 5 (Table 2). However, at 75% r.h. there were more arrested *T. castaneum* than *T. confusum* larvae in all treatments. The only significant comparison for relative humidity occurred for *T. castaneum* in treatment 2 (Table 2).

The number of live adult *T. confusum* ranged from $4.0 \pm 1.9\%$ to $41.0 \pm 15.4\%$ at 40% r.h. and $1.0 \pm 1.0\%$ to $14.0 \pm 4.3\%$ at 75% r.h., but there were no live adult *T. castaneum* at either relative humidity (Table 2). All comparisons between species were significant except for treatment 1, 75% r.h. At both 40% and 75% r.h., there was no difference in the percentage of live adult *T. confusum* between the Gentrol[®] treatment and the comparable rate of active ingredient in treatment 4, the “high” rate of experimental hydroprene formulation 202-080 (Table 2).

All dead *T. castaneum* exhibited gross morphological deformities, while the percentage of *T. confusum* with gross deformities ranged from $76.6 \pm 6.7\%$ to 100% at both relative humidity

Table 1

Experiment 1^a, analyses of variance for *Tribolium castaneum* and *Tribolium confusum* arrested in the larval stage after exposure on concrete treated with hydroprene, for live adults emerging from these larval exposures, and adults that died after emergence and exhibited either gross morphological deformities or wing deformities

Source	df	ms	F	P
<i>Arrested larvae</i>				
Species	1	10 609	38.2	<0.01
Relative humidity (r.h.)	1	2401	8.6	<0.01
Treatment	4	1783	6.4	<0.01
Species × r.h.	1	2500	9.0	<0.01
Species × treatment	4	735	2.7	0.04
R.h. × treatment	4	76	0.3	0.89
Species × r.h. × treatment	4	69	0.3	0.91
Error	80	278		
<i>Live adults</i>				
Species	1	4160	34.6	<0.01
Relative humidity (r.h.)	1	812	6.8	0.01
Treatment	4	485	4.0	<0.01
Species × r.h.	1	812	6.8	0.01
Species × treatment	4	485	2.7	<0.01
R.h. × treatment	4	216	1.8	0.14
Species × r.h. × treatment	4	216	1.8	0.14
Error	80	120		
<i>Gross deformities</i>				
Species	1	1633.0	59.2	<0.01
Relative humidity (r.h.)	1	138.0	5.0	0.03
Treatment	4	178.6	6.5	<0.01
Species × r.h.	1	138.0	5.0	0.03
Species × treatment	4	178.6	6.5	<0.01
R.h. × treatment	4	56.1	2.0	0.10
Species × r.h. × treatment	4	56.1	2.0	0.10
Error	80	27.6		
<i>Wing deformities</i>				
Species	1	23.0	59.2	<0.01
Relative humidity (r.h.)	1	0.1	0.1	0.81
Treatment	4	1.7	2.5	0.04
Species × r.h.	1	0.1	0.1	0.81
Species × treatment	4	1.7	2.5	0.04
R.h. × treatment	4	0.3	0.5	0.74
Species × r.h. × treatment	4	0.3	0.5	0.74
Error	80	0.7		

^a Five treatments, 1.9×10^{-3} mg [AI]/cm² Gentrol[®] (the labeled rate) or 0.85 (low rate) and 1.9×10^{-3} mg [AI]/cm² (high rate) of experimental hydroprene formulations 202-080 and 202-084, two levels of relative humidity (r.h.), 40% and 75%, two insect species, five replications.

Table 2

Experiment 1, percentage (mean \pm SE) of *Tribolium confusum* and *Tribolium castaneum* arrested in the larval stage, and the percentage of adults that emerged and lived (live adults) after exposure as late-instar larvae on concrete treated with 1.9×10^{-3} mg [AI]/cm² Gentrol[®] or 0.85 (low rate) and 1.9×10^{-3} mg [AI]/cm² (high rate) of experimental hydroprene formulations 202-080 and 202-084, at 40% and 75% relative humidity (r.h.)

Species	Larvae ^a		Live adults ^b	
	40% r.h.	75% r.h.	40% r.h. ^c	75% r.h.
<i>Gentrol</i> [®]				
<i>T. confusum</i>	14.0 \pm 4.3a	7.0 \pm 4.9b	4.0 \pm 1.9a ^d	1.0 \pm 1.0b
<i>T. castaneum</i>	29.0 \pm 10.4a	47.0 \pm 5.6a	0.0 \pm 0.0	0.0 \pm 0.0
202-080, low rate ^c				
<i>T. confusum</i>	40.0 \pm 2.9a	7.0 \pm 4.6b	41.0 \pm 15.4a	12.0 \pm 4.6ab
<i>T. castaneum</i>	2.0 \pm 1.2a	26.0 \pm 8.0a	0.0 \pm 0.0	0.0 \pm 0.0
202-080, high rate				
<i>T. confusum</i>	16.0 \pm 9.1a	17.0 \pm 10.5b	4.0 \pm 2.9b	4.0 \pm 2.9b
<i>T. castaneum</i>	32.0 \pm 9.8a	50.0 \pm 11.1a	0.0 \pm 0.0	0.0 \pm 0.0
202-084, low rate				
<i>T. confusum</i>	6.0 \pm 4.0a	8.0 \pm 2.5b	17.0 \pm 3.4ab	14.0 \pm 4.3a
<i>T. castaneum</i>	9.0 \pm 6.7a	20.0 \pm 9.4a	0.0 \pm 0.0	0.0 \pm 0.0
202-084, high rate				
<i>T. confusum</i>	11.0 \pm 6.0a	11.0 \pm 2.9b	27.0 \pm 12.7ab	5.0 \pm 2.7ab
<i>T. castaneum</i>	32.0 \pm 11.4a	60.0 \pm 9.6a	0.0 \pm 0.0	0.0 \pm 0.0

^a Means for arrested larvae within columns for each relative humidity that are followed by the same lower-case letters are not significantly different with respect to species ($P \geq 0.05$, Proc *t*-test, SAS Institute, 2001).

^b No live adult *T. castaneum* at either relative humidity; all means were significantly different with respect to species ($P < 0.05$) except for treatment 2, 75% r.h.

^c There were no significant differences between relative humidities for live adult *T. confusum* ($P \geq 0.05$).

^d For each relative humidity, the means for percentage of live adult *T. confusum* followed by the same letter are not significantly different with respect to treatment ($P \geq 0.05$, Waller–Duncan *k*-ratio *t*-test, SAS Institute, 2001).

^e For this treatment, there were more *T. castaneum* arrested in the larval stage at 75% than at 40% r.h. ($P < 0.05$, *t*-test); no other comparisons were significant for relative humidity ($P \geq 0.05$, Proc *t*-test, SAS Institute, 2001).

levels (Table 3). There were no differences in the percentages of *T. confusum* with gross deformities with respect to relative humidity when individual treatments were analyzed. Although the percentage of *T. confusum* with gross deformities was consistently lower in treatment 2 at both 40% and 75% r.h., there were no differences at either relative humidity among Gentrol[®] and the two experimental hydroprene formulations applied at the same rate (Table 3). The percentage of *T. confusum* with wing deformities ranged from $2.3 \pm 1.4\%$ to $15.7 \pm 6.6\%$ at 40% r.h. and 0.0% to $8.3 \pm 2.9\%$ at 75% r.h. (Table 3). The overall ANOVA indicated a treatment effect, but there was no difference in the percentage of adult *T. confusum* with wing deformities among the five treatments at either relative humidity.

Table 3

Experiment 1, percentage (mean \pm SE) of adult *Tribolium confusum* that died after emergence (dead adults), with gross morphological deformities or wing^a deformities only, 5 weeks after being exposed as late-instar larvae on concrete treated with 1.9×10^{-3} mg [AI]/cm² Gentrol[®] or 0.85 (low rate) and 1.9×10^{-3} mg [AI]/cm (high rate) of experimental hydroprene formulations 202-080 and 202-084, at 40% and 75% r.h.^b

Formulation/rate	Gross deformities		Wing deformities	
	40% r.h.	75% r.h.	40% r.h.	75% r.h.
1. Gentrol [®]	97.7 \pm 1.4a	96.8 \pm 2.1ab	2.3 \pm 1.4a	3.2 \pm 2.0a
2. 202-080, low rate	76.3 \pm 6.7b	89.6 \pm 3.2b	15.7 \pm 6.6a	7.6 \pm 3.6a
3. 202-080, high rate	96.3 \pm 2.5a	93.2 \pm 1.9ab	3.7 \pm 2.5a	6.8 \pm 1.9a
4. 202-084, low rate	85.8 \pm 3.7ab	91.7 \pm 2.9b	8.9 \pm 3.1a	8.3 \pm 2.9a
5. 202-084, high rate	91.7 \pm 4.2a	100 \pm 0.0a	3.8 \pm 2.5a	0 \pm 0.0a

^a All adult *T. castaneum* that died after they emerged were classified as having gross morphological deformities; none exhibited wing deformities only.

^b For each relative humidity, means for percentage of dead adults with gross morphological deformities or wing deformities, followed by the same letter, are not significantly different with respect to treatment ($P \geq 0.05$, Waller–Duncan k -ratio t -test, SAS Institute, 2001).

3.2. Experiment 2. Impact of environmental conditions on residual efficacy of hydroprene against *T. confusum*

Initial bioassays at week 0 were combined over storage environment, because they were all conducted at 27°C, 57% r.h. to provide baseline efficacy data similar to a treatment in which no residual data would be assessed or collected. These data were also eliminated from the statistical analysis, and each of the response variables was then analyzed in succession. Only bioassay time and formulation \times storage environment were significant with respect to the percentage of arrested larvae, while all main effects and interactions were significant for live adults (Table 4). All main effects and all but one interaction were significant with respect to the percentage of dead adults with gross morphological deformities, but no main effects were significant for wing deformities (Table 4).

The percentage of arrested larvae from the initial bioassays (week 0) on concrete treated with formulations 202-080 and 202-084 was $15.7 \pm 2.6\%$ and $21.2 \pm 2.6\%$, respectively. When data for each formulation were then analyzed at each bioassay interval, there were no differences among storage environments regarding the percentage of arrested larvae on concrete that had been treated with hydroprene formulation 202-080 (Table 5). However, at 6 weeks there were more arrested larvae on concrete that had been treated with formulation 202-084 and held at 32°C, 75% r.h. compared to 22°C, 40% and 75% r.h. There were fewer arrested larvae at 12 weeks than at 6 weeks on concrete treated with formulation 202-080 and held at 32°C, 40% r.h., and fewer arrested larvae at 12 weeks than at 6 weeks on concrete treated with formulation 202-084 and held at 32°C, 40% and 75% r.h., but no other comparison was significant (Table 5).

The percentage of live adults from the initial bioassays (week 0) on concrete treated with formulations 202-080 and 202-084 was $12.5 \pm 4.8\%$ and $0.5 \pm 0.3\%$, respectively. At week 6, there were fewer live adults on concrete treated with formulation 202-080 and held at 32°C, 40% r.h.

Table 4

Experiment 2^a, analyses of variance for *Tribolium confusum* arrested in the larval stage after exposure on concrete treated with hydroprene, for live adults emerging from these larval exposures, and for adults that died after emergence and exhibited either gross morphological deformities or wing deformities

Source	df	ms	F	P
<i>Arrested larvae</i>				
Formulation	1	7.8	0.1	0.71
Time	1	1757.8	32.2	<0.01
Treatment	3	102.0	1.9	0.14
Formulation × time	1	7.8	0.1	0.71
Formulation × treatment	3	226.1	4.2	<0.01
Time × treatment	3	31.1	0.6	0.64
Formulation × time × treatment	3	102.8	1.9	0.14
Error	64	54.5		
<i>Live adults</i>				
Formulation	1	3380.0	14.5	<0.01
Time	1	37 441.2	160.8	<0.01
Treatment	3	9740.0	41.9	<0.01
Formulation × time	1	1901.2	8.2	<0.01
Formulation × treatment	3	3773.3	16.2	<0.01
Time × treatment	3	2414.5	10.3	<0.01
Formulation × time × treatment	3	1631.2	5.9	<0.01
Error	64	232.6		
<i>Gross deformities</i>				
Species	1	16 744	56.7	<0.01
Relative humidity (r.h.)	1	16 224	54.9	<0.01
Treatment	4	8045	27.2	<0.01
Species × r.h.	1	2	0.1	0.93
Species × treatment	4	4156	14.1	<0.01
R.h. × treatment	4	2424	8.2	<0.01
Species × r.h. × treatment	4	1614	5.6	<0.01
Error	80	295		
<i>Wing deformities</i>				
Species	1	158	0.7	0.40
Relative humidity (r.h.)	1	549	2.4	0.12
Treatment	4	548	2.5	0.07
Species × r.h.	1	957	4.3	0.04
Species × treatment	4	62	0.3	0.84
R.h. × treatment	4	293	1.3	0.28
Species × r.h. × treatment	4	71	0.3	0.81
Error	80	223		

^a Concrete was treated with 1.9×10^{-3} mg [AI]/cm of experimental hydroprene formulations 202-080 and 202-084 and stored for 6 and 12 weeks at 22°C or 32°C, 40 or 75% r.h. (four treatment combinations, five replicates). Bioassays were conducted at the 6- and 12-week post-treatment intervals at 27°C, 57% r.h. to standardize conditions for exposures.

Table 5

Experiment 2^a, percentage (mean \pm SE) of *Tribolium confusum* arrested in the larval stage and percentage of adults that emerged and lived (live adults) after being exposed as late-instar larvae on concrete treated with 1.9×10^{-3} mg [AI]/cm² of experimental hydroprene formulations 202-080 and 202-084

Environment ^b	Larvae ^c		Live adults ^d	
	Week 6 ^e	Week 12	Week 6	Week 12
<i>Formulation 202-080</i>				
22°C, 40% r.h.	11.0 \pm 5.1a	1.0 \pm 1.0a	35.0 \pm 5.0a	72.0 \pm 14.8a
22°C, 75% r.h.	12.0 \pm 6.8a	0 \pm 0.0a	56.0 \pm 10.7aB	97.0 \pm 1.2aA
32°C, 40% r.h.	11.0 \pm 4.3aA	0 \pm 0.0aB	7.0 \pm 2.54bB	84.0 \pm 2.9aA
32°C, 75% r.h.	7.0 \pm 3.0a	0 \pm 0.0a	32.0 \pm 10.6aB	89.0 \pm 4.3aA
<i>Formulation 202-084</i>				
22°C, 40% r.h.	10.0 \pm 4.1b	0 \pm 0.0b	8.0 \pm 2.5bB	69.0 \pm 6.9aA
22°C, 75% r.h.	0 \pm 0.0c	0 \pm 0.0b	94.0 \pm 2.4a	93.0 \pm 4.3a
32°C, 40% r.h.	9.0 \pm 2.4abA	1.0 \pm 1.0bB	8.0 \pm 2.5bB	73.0 \pm 6.0aA
32°C, 75% r.h.	22.0 \pm 6.8aA	5.0 \pm 1.6aB	7.0 \pm 5.8b	16.0 \pm 9.2b

^aConcrete was stored at 22°C, 40% r.h.; 22°C, 75% r.h.; 32°C, 40% r.h.; or 32°C, 75% r.h. for 6 and 12 weeks before larvae were exposed, but the bioassays were conducted at 27°C, 57% r.h. to ensure optimum conditions for development of *T. confusum*.

^bMeans for arrested larvae or live adults within columns for each relative humidity that are followed by the same lower-case letters are not significantly different with respect to environment ($P \geq 0.05$, Waller–Duncan k -ratio t -test, SAS Institute, 2001).

^cThe percentage of arrested larvae from bioassays conducted at week 0 on concrete treated with formulations 202-080 and 202-084 was $15.7 \pm 2.6\%$ and $21.2 \pm 2.6\%$, respectively.

^dThe percentage of live adults from bioassays conducted at week 0 on concrete treated with formulations 202-080 and 202-084 was $12.5 \pm 4.8\%$ and $0.5 \pm 0.3\%$, respectively.

^eMeans for arrested larvae or live adults within rows for each formulation and environment that are followed by different capital letters are significantly different with respect to week 6 or week 12 ($P < 0.05$, Proc t -test, SAS Institute, 2001); when no capital letters appear means were not significantly different ($P \geq 0.05$).

compared with the other storage environments (Table 5). The number of live adults on concrete treated with formulation 202-084 and held at 22°C, 75% r.h. was 94.0 ± 2.4 , which was consistent with the fact that no arrested larvae were found in these bioassays. The number of live adults did not exceed 8.0 ± 2.5 in the other environments. At the 12-week bioassays, there were fewer adults on concrete that had been treated with formulation 202-084 and held at 32°C, 75% r.h., but no other differences were detected among the storage environments, and the percentage of live adults was generally much higher at 12 weeks compared with 6 weeks.

The percentage of dead adults with gross morphological deformities in the 6-week bioassays on concrete treated with formulation 202-080 ranged from $6.7 \pm 6.7\%$ to $68.7 \pm 6.6\%$, and there were more dead adults with gross morphological deformities in the bioassays on concrete that had been stored at 32, compared with 22°C (Table 6). There were no dead adults with morphological deformities in the 6-week bioassays on concrete treated with formulation 202-084 and stored at 22°C, 75% r.h., but the percentage of adults with gross deformities in the other three storage environments was $71.2 \pm 4.3\%$ or greater, with no significant differences among these three

Table 6

Experiment 2^a, percentage (mean \pm SE) of dead adult *Tribolium confusum*, as a percentage of the total number of dead emerged adults, with gross morphological deformities or wing deformities only, after being exposed as late-instar larvae on concrete treated with 1.9×10^{-3} mg [AI]/cm² of experimental hydroprene formulations 202-080 and 202-084

Environment ^b	Gross deformities ^c		Wing deformities ^d	
	Week 6 ^e	Week 12	Week 6	Week 12
<i>Formulation 202-080</i>				
22°C, 40% r.h.	10.7 \pm 6.6bc	0 \pm 0.0a	19.5 \pm 8.4a	15.6 \pm 15.6a
22°C, 75% r.h.	6.7 \pm 6.7c	0 \pm 0.0a	17.4 \pm 7.2a	0 \pm 0.0a
32°C, 40% r.h.	68.7 \pm 6.6aA	0 \pm 0.0aB	15.1 \pm 1.1aA	0 \pm 0.0aB
32°C, 75% r.h.	35.1 \pm 15.5abA	0 \pm 0.0a	16.1 \pm 7.2aA	0 \pm 0.0a
<i>Formulation 202-084</i>				
22°C, 40% r.h.	74.8 \pm 8.0aA	8.5 \pm 8.5bB	10.0 \pm 2.5abB	23.7 \pm 14.2aA
22°C, 75% r.h.	0 \pm 0.0b	0 \pm 0.0b	0 \pm 0.0b	0 \pm 0.0a
32°C, 40% r.h.	71.2 \pm 4.3aA	19.5 \pm 15.1bB	12.5 \pm 2.5aA	0 \pm 0.0aB
32°C, 75% r.h.	87.2 \pm 6.6a	79.0 \pm 5.7a	4.7 \pm 5.8ab	7.7 \pm 6.4a

^a The treated concrete was stored at one of four storage environments: 22°C, 30% r.h.; 22°C, 75% r.h.; 32°C, 30% r.h.; or 32°C, 75% r.h. for 6 and 12 weeks before larvae were exposed, but bioassays were conducted at 27°C, 57% r.h. to ensure optimum conditions for development of *T. confusum*.

^b Means for dead adults with gross morphological deformities or dead adults with wing deformities only in columns for each relative humidity that are followed by the same lower-case letters are not significantly different with respect to environment ($P \geq 0.05$, Waller–Duncan *k*-ratio *t*-test, SAS Institute, 2001).

^c The percentage of dead adults with gross morphological deformities from bioassays conducted at week 0 on concrete treated with formulations 202-080 and 202-084 was $77.7 \pm 7.4\%$ and $94.4 \pm 2.2\%$, respectively.

^d The percentage of dead adults with wing deformities only from bioassays conducted at week 0 on concrete treated with formulations 202-080 and 202-084 was $9.3 \pm 3.7\%$ and $3.2 \pm 1.3\%$, respectively.

^e Means for dead adults with gross morphological deformities or dead adults with wing deformities only within rows for each formulation and environment that are followed by different capital letters are significantly different with respect to week 6 or week 12 ($P < 0.05$, Proc *t*-test, SAS Institute, 2001); when no capital letters appear means were not significantly different ($P \geq 0.05$).

environments (Table 6). At 12 weeks, there were no dead adults with gross deformities in bioassays with concrete treated with formulation 202-080. The percentage of dead adults with gross deformities in bioassays with concrete treated with formulation 202-084 and held at 32°C, 75% r.h. was $79.0 \pm 5.7\%$, which was much greater than the corresponding percentages in the other three storage environments. In bioassays with formulation 202-080, 32°C, and with formulation 202-084, 22 and 32°C, 40% r.h., there was a drastic decline in the percentage of dead adults with gross deformities at week 12 compared with week 6 (Table 6).

The percentage of dead adults with wing deformities only from the initial bioassays on concrete treated with formulations 202-080 and 202-084 was $9.3 \pm 3.7\%$ and $3.2 \pm 1.3\%$, respectively. At the 6-week bioassays on concrete treated with formulation 202-080, the percentage of adults with wing deformities did not exceed $19.5 \pm 8.4\%$, with no differences among the storage environments (Table 6). There were some differences in the 6-week bioassays for formulation 202-084, primarily because there were no adults with wing deformities in the bioassays of concrete held at 22°C,

75% r.h.. A few adults with wing deformities were found in bioassays with concrete held at 32°C, 75% r.h., which was unusual because of the large percentage of adults with gross deformities. At 12 weeks, no dead adults exhibited wing deformities in bioassays with concrete treated with formulation 202-080, except for one replicate held at 22°C, 40% r.h., which resulted in a significant difference between week 6 and week 12 for concrete held at 32°C. There was considerable variation in the bioassays at week 12 for concrete treated with formulation 202-084, but no significant differences among the storage environments (Table 6).

3.3. Experiment 3. Residual susceptibility of *T. confusum* to hydroprene on concrete repeatedly covered with flour

Main effects formulation and bioassay time were significant with respect to the percentage of arrested larvae, but neither the main effect exposure condition nor any of the interactions were significant, with the exception of formulation \times bioassay time (Table 7). Differences in the percentage of live adults were significant for bioassay time, but not formulation or exposure condition, and few interactions were significant (Table 7). The percentage of dead adults with wing deformities differed significantly with formulation, but not bioassay time or exposure conditions, and no interaction was significant (Table 7). Because there was no significant difference with respect to exposure condition for these three variables, data were combined for further analysis. The percentage of dead adults with gross morphological deformities was significantly different among all main effects and interactions, except formulation \times exposure condition (Table 7).

In the 0-week bioassays, there were more arrested larvae on concrete that had been treated with formulation 202-084 compared with formulation 202-080. However, at 6 and 12 weeks there were no arrested larvae in bioassays conducted with either formulation, except for one replicate in formulation 202-084, 6-week bioassays (Table 8). The percentage of live adults in bioassays conducted at week 0 was 6.0 ± 2.0 and 1.7 ± 0.8 for formulations 202-080 and 202-084, respectively, but in the 6- and 12-week bioassays the percentage of live adults increased sharply for both formulations (Table 8). There was no difference in the percentage of dead adults with wing deformities among bioassays of formulation 202-080 and there were no dead adults with wing deformities for bioassays conducted at weeks 6 and 12. However, in bioassays with formulation 202-084, the percentage of dead adults with wing deformities increased for the 6- and 12-week bioassays, and was greater than the corresponding percentages for formulation 202-080.

The percentage of dead adults in bioassays from week 0 of concrete treated with formulation 202-080 was lowest at 32°C, 75% r.h., and there were no dead adults with gross morphological deformities in bioassays with this formulation at 6 and 12 weeks (Table 9). The percentage of dead adults with gross morphological deformities from bioassays conducted with formulation 202-084 at week 0 ranged from 91.8% to 94.7%, with no difference among exposure conditions. A greater percentage of dead adults with gross deformities occurred in bioassays from week 6 at 27°C, 57% r.h. compared to the other conditions, but there were no dead adults with gross deformities in bioassays at weeks 6 and 12 from other exposure conditions, except for one replicate at 6 weeks, 27°C, 57% r.h. Because of the large number of 0 values at weeks 6 and 12, the percentage of adults with gross deformities was always greatest in bioassays from week 0 (Table 9).

Table 7

Experiment 3^a, analyses of variance for *Tribolium confusum* arrested in the larval stage after exposure on concrete treated with hydroprene, for live adults emerging from these larval exposures, and for adults that died after emergence and exhibited either gross morphological deformities or wing deformities

Source	df	ms	<i>F</i>	<i>P</i>
<i>Arrested larvae</i>				
Formulation	1	585.2	14.7	<0.01
Time	2	2062.7	51.8	<0.01
Treatment	3	4.1	0.1	0.95
Formulation × time	2	553.7	13.9	<0.01
Formulation × treatment	3	14.1	0.3	0.77
Time × treatment	6	3.3	0.8	0.99
Formulation × time × treatment	6	14.9	0.4	0.89
Error	96	39.8		
<i>Live adults</i>				
Formulation	1	13.3	0.1	0.75
Time	2	89561.8	715.9	<0.01
Treatment	3	123.9	1.0	0.40
Formulation × time	2	1952.2	15.6	<0.01
Formulation × treatment	3	190.6	1.5	0.21
Time × treatment	6	423.2	3.4	<0.01
Formulation × time × treatment	6	282.4	2.2	0.04
Error	96	125.1		
<i>Wing deformities</i>				
Formulation	1	2213.9	12.2	<0.01
Time	2	302.9	1.7	0.19
Treatment	3	321.1	1.8	0.16
Formulation × time	2	897.7	4.9	<0.01
Formulation × treatment	3	449.5	2.5	0.07
Time × treatment	6	319.2	1.8	0.12
Formulation × time × treatment	6	311.7	1.7	0.14
Error	96	182.0		
<i>Gross deformities</i>				
Formulation	1	2219.9	33.4	<0.01
Time	2	93348.5	1068.2	<0.01
Treatment	3	445.8	5.1	<0.01
Formulation × time	2	388.2	3.9	0.02
Formulation × treatment	3	214.8	2.5	0.07
Time × treatment	6	204.43	2.3	0.04
Formulation × time × treatment	6	403.19	4.6	<0.01
Error	96	87.4		

^a Larvae were exposed with 500 mg of wheat flour at one of four temperature-relative humidity (r.h.) combinations: 27°C, 57% r.h.; 27°C, 75% r.h.; 32°C, 57% r.h., or 32°C, 75% r.h., and procedures were repeated at 6 and 12 weeks after treatment.

Table 8

Experiment 3^a, percentage (mean \pm SE) of *Tribolium confusum* arrested in the larval stage, percentage of individual beetles that had either emerged and lived after being exposed as late-instar larvae on concrete treated with 1.9×10^{-3} mg [AI]/cm² of experimental hydroprene formulations 202-080 and 202-084, or had died after emergence and exhibited wing deformities^{b,c}

Week	Larvae	Live adults	Dead adults with wing deformities
<i>Formulation 202-080</i>			
0	6.0 \pm 1.1a	6.0 \pm 2.0c	3.4 \pm 1.1a
6	0 \pm 0.0b	91.0 \pm 1.8a	0 \pm 0.0a
12	0 \pm 0.0b	79.5 \pm 3.3b	0 \pm 0.0a
<i>Formulation 202-084</i>			
0	19.0 \pm 3.0a	1.7 \pm 0.8c	3.1 \pm 1.4b
6	0.2 \pm 0.2b	78.5 \pm 4.7b	16.6 \pm 6.8a
12	0 \pm 0.0b	94.2 \pm 2.0a	19.0 \pm 9.1a

^a Larvae were exposed with 500 mg of wheat flour at one of four temperature-relative humidity (r.h.) combinations: 27°C, 57% r.h.; 27°C, 75% r.h.; 32°C, 57% r.h.; or 32°C, 75% r.h., and procedures were repeated at 6 and 12 weeks after treatment.

^b There were no significant differences among exposure conditions (temperature-relative humidity combinations) with respect to the percentage of arrested larvae, the percentage of live adults, or the percentage of dead adults with wing deformities only. Data combined for analysis of bioassay week.

^c For each formulation, means for larvae, live adults, and dead adults with wing deformities, followed by the same letter, are not significantly different ($P \geq 0.05$, Waller–Duncan *k*-ratio *t*-test, SAS Institute, 2001).

4. Discussion

Tribolium castaneum was much more susceptible than *T. confusum* to both Gentrol[®] and the two experimental hydroprene formulations 202-080 and 202-084. Similar results also occurred when *T. castaneum* and *T. confusum* were exposed on concrete treated with different concentrations of Gentrol[®] (Arthur, 2001), though the differences were not as clear as in experiment 1. *Tribolium castaneum* was also more susceptible than *T. confusum* to a volatile formulation of hydroprene dispensed from a wick instead of direct application to a treated surface (Arthur, 2003). Although these tests with hydroprene clearly established a difference between the two species, differential susceptibility of *T. castaneum* and *T. confusum* may vary depending on insecticide, formulations, and application techniques (Arthur, 2000b).

Residual efficacy of hydroprene on a concrete surface may be related to the temperature and relative humidity at which the concrete is held or stored. In experiment 2 there was an apparent loss of efficacy or inactivation of hydroprene after 6 weeks of storage at 22°C, 75% r.h. compared with the combinations of 22°C, 40% r.h., 32°C, 40% r.h., and 32°C, 75% r.h., particularly with formulation 202-084. It was obvious that a loss of efficacy had occurred on the concrete that had been held at 22°C, 75% r.h. because few deleterious effects were produced in the bioassays at 6 weeks, but the reasons for this loss of efficacy are not clear because there were no consistent trends. When the treated concrete was kept clean and stored without any contaminant on the surface, the hydroprene still had residual activity after 6 weeks of storage at all temperature-relative humidity combinations except 22°C, 75% r.h., but very little control occurred after

Table 9

Percentage (mean \pm SEM) of dead *Tribolium confusum* adults that had emerged from pupae and that exhibited gross morphological deformities, 5 weeks after being exposed as late-instar larvae on concrete treated with 1.9×10^{-3} mg [AI]/cm² of experimental hydroprene formulations 202-080 and 202-084. Larvae were exposed with 500 mg of wheat flour at one of four temperature-relative humidity (r.h.) combinations: 27°C, 57% r.h.; 27°C, 75% r.h.; 32°C, 57% r.h.; or 32°C, 75% r.h.^a; and procedures were repeated at 6 and 12 weeks after treatment

Environment ^b	Week 0	Week 6	Week 12
<i>Formulation 202-080</i>			
27°C, 57% r.h.	97.5 \pm 2.5a	0 \pm 0.0a	0 \pm 0.0a
27°C, 75% r.h.	94.4 \pm 4.1ab	0 \pm 0.0a	0 \pm 0.0a
32°C, 57% r.h.	91.7 \pm 4.2ab	0 \pm 0.0a	0 \pm 0.0a
32°C, 75% r.h.	83.1 \pm 3.1b	0 \pm 0.0a	0 \pm 0.0a
<i>Formulation 202-084</i>			
27°C, 57% r.h.	94.7 \pm 5.3aA	37.5 \pm 14.0aB	0 \pm 0.0aC
27°C, 75% r.h.	97.6 \pm 1.5a	5.0 \pm 5.0b	0 \pm 0.0a
32°C, 57% r.h.	91.8 \pm 4.4a	0 \pm 0.0b	0 \pm 0.0a
32°C, 75% r.h.	97.5 \pm 2.5a	0 \pm 0.0b	0 \pm 0.0a

^a Means for dead adults with gross morphological deformities or dead adults with wing deformities only in columns for each bioassay time that are followed by the same lower-case letters are not significantly different with respect to exposure condition ($P \geq 0.05$, Waller–Duncan k -ratio t -test, SAS Institute, 2001).

^b Means for dead adults with gross morphological deformities within rows for each formulation and exposure condition that are followed by different capital letters are significantly different with respect to week 6 or week 12 ($P < 0.05$, Proc t -test, SAS Institute, 2001); when no capital letters appear means were not significantly different ($P \geq 0.05$).

12 weeks except in those concrete arenas stored at 32°, 75% r.h. Perhaps the hydroprene is more volatile at higher temperatures, and therefore more available to insects that come into contact with the residues. The cooler temperature of 22°C may have also bound up the residues and reduced some of this volatility so that the efficacy of the residues was reduced.

In experiment 3 there was little or no residual activity of hydroprene, probably because bioassays were repeated on the concrete arenas, which had been covered with the flour used in the bioassays at week 0. These results were in marked contrast to results for experiment 2 in which the concrete disks were stored clean and different sets of arenas were used in the residual bioassays. The presence of accumulated flour and other material also caused a reduction in residual efficacy of cyfluthrin when adult *T. castaneum* were exposed on treated concrete (Arthur, 2000a). Similarly, survival of both adult *T. castaneum* and *T. confusum* increased when they were provided with a flour food source either during or after exposure to diatomaceous earth (Arthur, 2000c) and to a particle film dust (Arthur and Puterka, 2002). The flour could have absorbed some of the hydroprene from the treated surface, thereby affecting residual efficacy. However, the flour was necessary for these tests using *T. confusum* larvae because without the flour food source normal development to the adult stage would have been compromised. If flour or other material is allowed to accumulate on concrete treated with hydroprene, a loss of residual efficacy will be the likely result, and the surface may have to be cleaned and hydroprene application repeated to achieve control.

Many studies reviewed by Oberlander et al. (1997) document persistence and residual control lasting for anywhere from 6 to 18 months when IGRs are applied as protectants of stored grains. Most of these tests were conducted by storing treated grains for various time intervals at a single temperature and moisture content or equilibrium relative humidity, conducting bioassays at various post-treatment intervals by exposing eggs of stored-product insects, and measuring inhibition of F₁ progeny production compared to untreated controls. Samson et al. (1990) showed a negative effect on susceptibility of *Rhyzopertha dominica* (F.), the lesser grain borer, to fenoxycarb on wheat and diflubenzuron on maize in conditions where equilibrium relative humidity was high, but did not find any consistent effects of equilibrium relative humidity on insecticidal activity. IGRs are very persistent on stored grains and structural surfaces, with slow loss of residues with time, as shown by White (1986) in studies with fenoxycarb. There are no comparable residual data for hydroprene on a treated surface, so it is difficult to compare the results of this study with related research. However, in the current studies in which hydroprene was applied to concrete, residual control lasted for only a matter of weeks, as compared to the much longer time periods reported for tests on stored grains. This loss of activity is probably related to the characteristics of concrete, which is a porous surface. Many studies with stored-product insects document reduced efficacy of insecticides applied as water-based sprays on concrete compared to other surfaces (Arthur, 1994).

With the increased emphasis on integrated pest management (IPM) programs in stored products and the search for reduced-risk low toxicity insecticides, IGRs may receive increased use as residual surface treatments in mills, processing plants, and food storage facilities. The results of these studies show that there are physical and biological factors which may affect insecticidal efficacy of IGRs such as hydroprene, which must be considered in evaluation of these insecticides for inclusion into insect management programs. Relative humidity in particular seems to directly impact the efficacy of hydroprene.

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